NAVAL SHIP RESEARCH AND DEVELOPMENT CENTER

Washington, D.C. 20007

NAVAL SHIP RESEARCH AND DEVELOPMENT LABORATORY
Annapolis, Maryland 21402

DEEP OCEAN MICROBIOLOGICAL STUDIES - PART I - PHYSIOLOGICAL INTERACTION OF HYDROSTATIC AND OSMOTIC PRESSURE

By
E. C. Fischer and G. L. Liberatore

DEPARTMENT OF APPLIED SCIENCE

RESEARCH AND DEVELOPMENT REPORT

November 1970

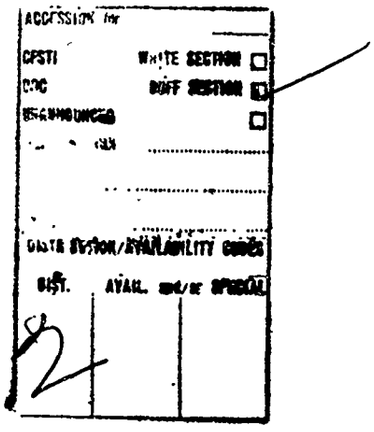
Report 3233

Deep Ocean Microbiological Studies, Part I - Physiological Interaction of Hydrostatic and Osmotic Pressure
Report 3233

16

The Naval Ship Research and Development Center is a U.S. Navy center for laboratory effort directed at achieving improved sea and air vehicles. It was formed in March 1967 by merging the David Taylor Model Basin at Carderock, Maryland and the Marine Engineering Laboratory at Annapolis, Maryland.

Naval Ship Research and Development Center
Washington, D.C. 20007



NDW-NSRDC 3960/44 (10-67)

DEEP OCEAN MICROBIOLOGICAL STUDIES
PART I - PHYSIOLOGICAL INTERACTION OF HYDROSTATIC AND OSMOTIC PRESSURE

By
E. C. Fischer and G. L. Liberatore

November 1970 9-42 Report 3233

ABSTRACT

High hydrostatic compression exerts depressive physiological effects on various microorganisms, including protozoans. In this report, experimental results are presented which show that some effects of high hydrostatic pressure are negated by increased osmotic pressure of the media (with salt or sucrose). In water with a salinity of 35 parts per thousand or osmotically equivalent sucrose concentration, the effects of 525 kilograms per centimeter square pressure (equivalent to an ocean depth of $\sim$17,000 feet) were diminished. Explanations for this phenomenon are discussed in relation to this new finding of osmotic protection.

ADMINISTRATIVE INFORMATION

This work is part of Task Area SF 11 552 101, Task 12874, Work Unit 1-937-101, as described in the 1 May 1970 Program Summary. This report is an adjunct to a previous publication issued in June 1967 as Progress Report 1, "Deep Ocean Microbiological Studies," at the Naval Applied Science Laboratory, Brooklyn, New York. The technical staff and facilities involved in this work were transferred in June 1970 to NAVSHIPRAND-LAB, Annapolis, where this program is being continued.

TABLE OF CONTENTS

NAVAL SHIP RESEARCH AND DEVELOPMENT LABORATORY

DEEP OCEAN MICROBIOLOGICAL STUDIES PART I - PHYSIOLOGICAL INTERACTION OF HYDROSTATIC AND OSMOTIC PRESSURE

By

E. C. Fischer and G. L. Liberatore

INTRODUCTION

Hydrostatic pressure equivalent to that of the deep ocean is one environmental factor which affects the physiological activities of organism living on or within the sea floor. Hydrostatic compression has been used as a tool in investigations of cellular phenomena such as pinocytosis,[1] amoeboid movement,[2] and sol-gel equilibria[3] in protozoans. However, most work in high pressure biology has been limited to the effects of hydrostatic compression on the growth and metabolism of bacteria.[4,5] The generally depressive effects of compression can be reduced or counteracted by increased incubation temperatures.[6,7] This report describes a similar beneficial effect induced by increased osmotic pressure of the hydrostatically compressed medium. This interaction was discovered during the development of techniques for the long-term culture of ciliated protozoans at high hydrostatic pressures, and may be a key to a more general biophysical principle which accounts for active metabolism of deep sea and ocean floor microorganisms.

MATERIALS AND METHODS

Axenic cultures of the euryhaline ciliate Glaucoma chattoni were maintained in an autoclaved crude medium containing 0.25% proteose peptone, 0.25% dextrose, and 0.2% yeast extract, pH 7.30. In long-term growth experiments, the osmotic concentration of the medium was varied by the addition of natural or synthetic seawater to a maximum salinity of 35 parts per thousand while maintaining the nutrient content constant. Inoculated media were aseptically drawn into glass syringes and injected with a sterile helium-oxygen gas mixture through a serum cap placed over the syringe

[1] Superscripts refer to similarly numbered entries in the Technical References at the end of the text.

tip. The gas mixture, imparting approximately 30 ppm* oxygen (= 21 microliters per milliliter) satisfied the aerobic requirements of the ciliates. Syringe cultures were compressed hydrostatically in steel pressure vessels and incubated at 19° C. Growth curves were formulated by electronic cell counting following decompression of the cultures as previously described.[8] The immediate morphological effects of compression and decompression were followed visually in a high pressure optical absorption cell (Aminco) and inverted microscope combination. The specimen chamber consisted of a small glass ring fused to the lower window and covered with a Teflon** membrane. The osmotic pressure of the compressed media, used in these short term studies, was increased by the incorporation of seawater (salinity of 35 parts per thousand) or an osmotically equivalent sucrose solution (24%, osmotically equivalent to seawater of salinity 35 parts per thousand.) Aseptic procedure was not used in those experiments utilizing the optical cell because of the short duration of the studies.

RESULTS

The growth rate of ciliate cultures in media without added seawater was inhibited progressively by increasing hydrostatic compression (Figure 1). At 210 kg/cm^2 (~3000 psi) multiplication was inhibited completely, but cell death did not occur. Following decompression after 100 hours exposure at this pressure, viable populations closely approximating those of the original inocula were counted. It was also found that cultures held at 210 kg/cm^2 pressure for 50 hours, then decompressed to lower pressures (either to atmospheric pressure or 70 kg/cm^2), began log phase growth immediately, and followed the growth pattern of cultures compressed initially at the same pressure levels. The progressive inhibition of growth rate by increasing hydrostatic pressure as compared with the control cultures at atmospheric pressure was diminished by increased salinity of the medium. This is indicated by the negative trend resulting from a comparison of the extent of inhibition of 70 kg/cm^2 pressure at various increasing salinities (Figure 2). This diminishing inhibition due to increased salinity is a protective effect which was demonstrated visually and quickly in the optical pressure cell. Ciliary activity was slowed immediately following compressions as low as 70 kg/cm^2 when nonsaline medium was used. Ciliates compressed at 350 kg/cm^2 for 30 minutes in nonsaline medium

*Abbreviations used in this report are from the GPO Style Manual, 1967, unless otherwise noted.

**Registered trademark of E. I. DuPont de Nemours Inc.

exhibited complete loss of motility (ciliary inactivation), sphering, the formation of large internal vacuoles, and increased cell volume (Figure 3B). Following rapid decompression, these spherical, enlarged organisms were often observed to burst or cytolyse. Identical ciliate cultures, when compressed at these pressure levels in media with a salinity of 35 parts per thousand, exhibited none of the above effects. At an even higher pressure (525 kg/cm^2), the cultures appeared normal in morphology and activity (Figure 3A) as did cultures of the marine ciliate Uronema marinum. In media with increased osmolarity due to sucrose addition, and compressed to 525 kg/cm^2, ciliates appeared normal in morphology with only slight loss of motility (ciliary activity).

DISCUSSION

The loss of motility (ciliary activity or amoeboid movement) in protozoans which were compressed hydrostatically has been historically associated with the sphering phenomenon,[2, 9, 10] which in turn has been attributed to the solational effect of high pressure on protoplasmic gels.[3, 11] The flexible and thick pellicle of certain ciliates reduced the sphering, thus indicating a structural resistance to form change.[12] The evidence presented here clearly indicates that hyperosmotic media afford protection to the organisms at high hydrostatic pressure.

It is reasonable to assume that pressurization at high levels appears to cause a breakdown of the water balance mechanisms of the ciliates when they are compressed in a hypoosmotic medium. The responses of ciliates compressed to 350 kg/cm^2 pressure indicate a net influx of water. The reported expansion of the pellicle and cessation of contractile vacuole activity of suctorians following compression[13] is additional evidence of water influx. Since ciliates are hyperosmotic to media without added seawater or sucrose (as was shown by the existence of water-eliminating contractile vacuoles), the pressure-induced water influx may be due to an inhibitory effect on contractile vacuolar activity probably involving sol-gel equilibria. The latter also diminishes ciliary activity. These effects obviously exert additional physiological stresses on the organisms, and are responsible for the depression of growth rate as shown in Figure 1. When these ciliates are compressed in seawater in which they are hypoosmotic or isoosmotic because of their euryhaline nature and the importance of the contractile vacuole activity is diminished, the above mentioned pressure-induced effects are not seen, even at pressures as high as 525 kg/cm^2. Thus, the reduced osmotic stresses could result in the improvement of growth rate in saline media at high pressures. The fact that increased osmolarity brought about by the addition of a nondissociating compound, such as sucrose, also counterbalances the hydrostatically-induced stress, indicates that stabilization of the cell integrity is not primarily a function of specific salt activity, but largely an osmotic one. Marine protozoa, which are isoosmotic

or only slightly hypoosmotic to seawater, and in which the contractile vacuole is of limited importance in osmotic balance, should show marked resistance to pressure-induced effects. Indeed, the marine ciliate, Uronema marinum showed no physiological changes when compressed to 525 kg/cm^2 in seawater medium.

Similar findings to those described here (antagonism between osmotic and hydrostatic pressure) have been found with coliform bacteria and deep ocean vibrios cultured at high hydrostatic pressure. The relationship between osmotic and hydrostatic pressure may involve a universal principle affecting the survival and multiplication of microorganisms in the deep ocean, and tissue cells under compression. These findings will be reported later as Part II of this report.

CONCLUSION

From the experimental evidence and inferences noted in the discussion, it is concluded that:

- Growth of ciliates in hypoosmotic media is progressively inhibited by increasing hydrostatic pressure.

- Growth inhibition by hydrostatic pressure is reduced by increasing the osmotic pressure of the media, either by salts or sugars.

- High hydrostatic pressure causes a breakdown of the water balance mechanism since there is a marked net influx of water into the ciliate.

The results implicate osmotic protection at high hydrostatic pressure as the basis for survival and multiplication of microorganisms in the deep ocean.

FUTURE WORK

Additional evidence concerning the interaction of osmotic and hydrostatic pressure on coliform bacteria and deep ocean sedimentary vibrios is being completed, and will be forwarded as Part II of this report. The relationship of the above interaction to bacterial metabolism, in particular the gas metabolism at high hydrostatic pressure, will be emphasized.

TECHNICAL REFERENCES

1 - Zimmerman, A. M. and R. C. Rustad; J. Cell. Bio., Vol. 25, 1965, p. 397

2 - Marsland, D. A.; Intern. Rev. Cytol., Vol. 6, 1956, p. 199

3 - Landau, J. V., A. M. Zimmerman, and D. A. Marsland; J. Cell and Comp. Physiol., Vol. 44, 1954, p. 211

4 - Zo Bell, C. E. and F. Johnson; J. Bacteriol., Vol. 57, 1949, p. 179

5 - Zo Bell, C. E. and A. Cobet; J. Bacteriol., Vol. 87, 1964, p. 710

6 - Rutberg, L.; Acta Pathol. Microbiol. Scand., Vol. 61, 1964, p. 91

7 - Heden, C., Bacteriol. Rev., Vol. 28, 1964, p. 14

8 - Fischer, E. C.; NASL Progress Rept. 1, 12 June 1967

9 - Kitching, V.; J. Exp. Biol., Vol. 34, 1957, p. 494

10 - Byrne, J. and D. A. Marsland; J. Cell and Comp. Physiol., Vol. 65, 1965, p. 277

11 - Landau, J. V. and L. Thibodeau; Exp. Cell Res., Vol. 27, 1962, p. 591

12 - Asterita, H. and D. A. Marsland; J. Cell and Comp. Physiol., Vol. 58, 1961, p. 49

13 - Kitching, V.; J. Exp. Biol., Vol. 31, 1954, p. 56

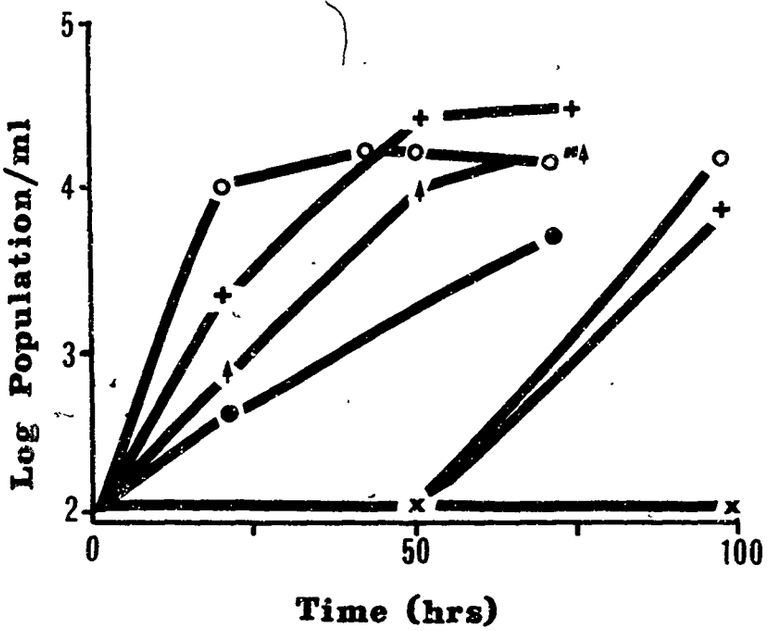


Figure 1
Growth of G. chattoni at Various Pressures (o = Atmospheric Control; + = 70 kg/cm^2; ↑= 140 kg/cm^2 ● 175 kg/cm^2; X = 210 kg/cm^2). In Some Experiments Pressure was Released After 50 Hours Compression at 210 kg/cm^2

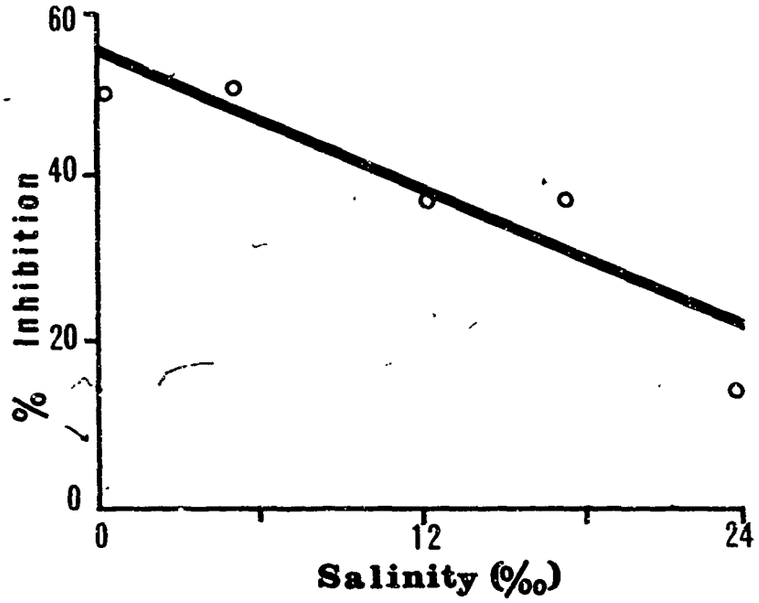


Figure 2
Reduction of the Inhibitive Effect of Hydrostatic Compression on Ciliate Growth by Increased Salinity of the Medium. Percent Inhibition is the Percent Decrease in the Total Cell Populations at 70 kg/cm^2 Pressure with Reference to Control Cultures at Atmospheric Pressure

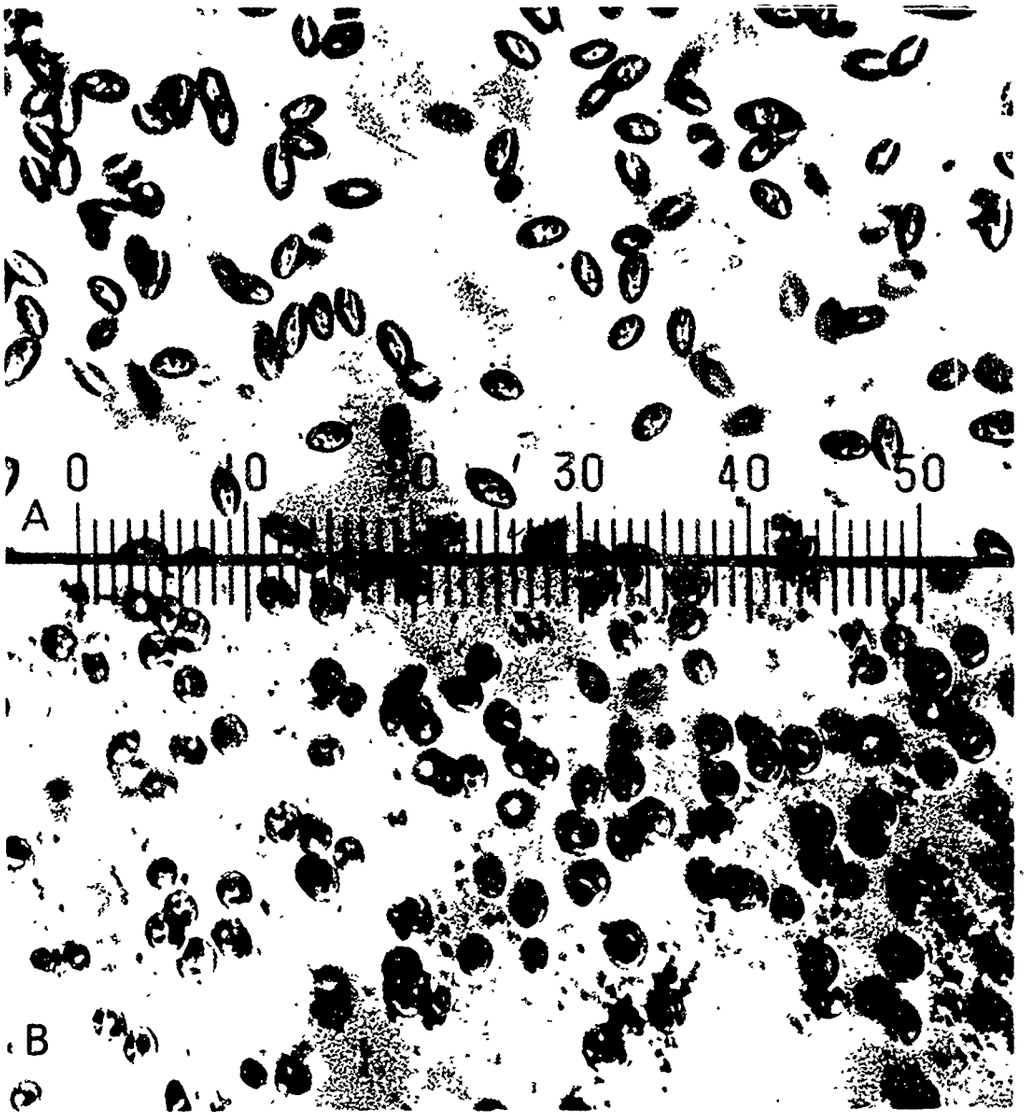


Figure 3
Morphology of Ciliates Photographed at High Hydrostatic Pressures; (A) G. chattoni in Seawater at 525 kg/cm^2 Pressure Showing Normal Morphology and Activity; (B) G. chattoni Culture in Medium without Added Seawater at 350 kg/cm^2 Pressure. The Increased Number of Ciliates in the Field of View is due to Ciliary Inactivation and Subsequent Sedimentation into the Optical Plane. One Division = 28 Microns.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

Naval Ship Research and Development Laboratory Annapolis, Maryland 21402	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

Deep Ocean Microbiological Studies, Part I - Physiological Interaction of Hydrostatic and Osmotic Pressure

4 DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5 AUTHOR(S) *(First name, middle initial, last name)*

E. C. Fischer and G. L. Liberatore

6 REPORT DATE	7a. TOTAL NO OF PAGES	7b. NO OF REFS
November 1970	13	13

8a CONTRACT OR GRANT NO	9a. ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO SF 11 552 101	3233
c. Task 12874	9b OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d Work Unit 937-101	9-42

10 DISTRIBUTION STATEMENT

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commander, Naval Ship Systems Command (SHIPS 00V1K) Washington, D. C. 20360

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	NAVSHIPS (SHIPS 00V1K)

13 ABSTRACT

High hydrostatic compression exerts depressive physiological effects on various microorganisms, including protozoans. In this report, experimental results are presented which show that some effects of high hydrostatic pressure are negated by increased osmotic pressure of the media (with salt or sucrose). In water with a salinity of 35 parts per thousand or osmotically equivalent sucrose concentration, the effects of 525 kilograms per centimeter square pressure (equivalent to an ocean depth of ~17,000 feet) were diminished. Explanations for this phenomenon are discussed in relation to this new finding of osmotic protection.

(Authors)

DD FORM 1 NOV 65 1473 (PAGE 1)

0102 014 6600

UNCLASSIFIED

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Hydrostatic pressure						
Deep ocean organisms						
Cellular phenomena						
Protozoans						
Osmotic pressure						
Compression						
Decompression						
Seawater						
Motility						